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NATURE OF THE NEGATIVE ELLIPTICITY OF HUMAN FETAL HEMOGLOBIN IN THE 280 nm REGION

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Summary

The uv circular dichroism (CD) spectra of aquomet hemoglobins A and F were followed to monitor their R→T conformational change. Titration studies with inositol hexaphosphate (IHP) for both adult and fetal hemoglobin showed identical total ellipticity changes although HbF was found to possess an inherently negative ultraviolet CD spectrum. By monitoring changes in the protein portion of the molecule, a dissociation constant for IHP of 16 μM was obtained for HbF. Chemical modification of HbF was found to leave the negative ellipticity unperturbed relative to native HbF. The results suggest that the negative ellipticity seen for stripped aquomet HbF is not due to a T conformation, but rather to an amino acid substitution in the γ chain of HbF.

Introduction

The presence of increased levels of fetal hemoglobin (HbF) in sickle cell anemia patients is known to alter the sickling properties of the hemoglobin molecule (HbS), possibly due to hybrid hemoglobin formation, to HbF adopting the deoxy (T) quaternary conformation more readily than HbS or to the different solubility properties found for deoxy HbF (1-3). Circular dichroism studies by Wind, et al. (4) revealed that by simply lowering the pH of the hemoglobin solution aquomet HbF adopts the T conformation, and they have postulated that the difference between fetal and adult hemoglobin is due to the relative stabilities of the R and T conformation. Earlier ORD (optical rotatory dispersion) studies by Li and Johnson (5) also reported that oxy HbF adopted an altered conformation at low pH and that increasing the pH resulted in a uv ORD spectrum which indicated an approach

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to a normal conformation. Li and Johnson attributed the differences to a substitution of a tryptophan for a tyrosine residue at position 130 of the non-α chain of HbF(5). In this report evidence is presented which indicates that the negative ellipticity seen for HbF is not due to the increased tendency of fetal hemoglobin to develop the T conformation but rather to the amino acid substitution of tryptophan for tyrosine.

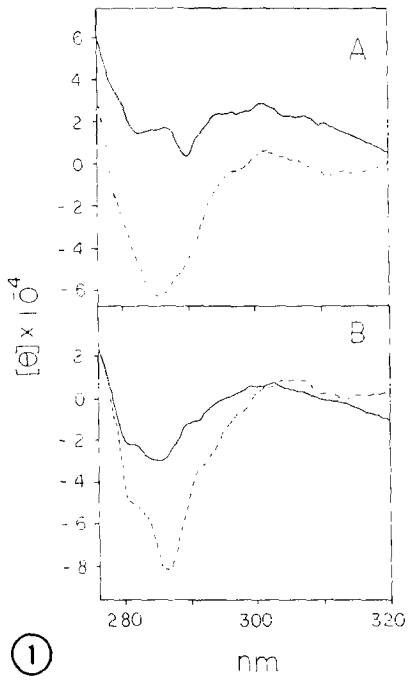
Methods

Hemoglobin A was purified from adult blood as described by Huisman (6). Hemoglobin F was purified by CM Sephadex chromatography from cord blood hemolysates which were obtained from Dr. T. H. J. Huisman. Met hemoglobin was prepared by addition of a 1.5 molar excess of $K_3Fe(CN)_6$ to the oxy Hb sample which was buffered in 50 mM tris, 0.1 M NaCl at pH 7.5. The sample was stripped of organic phosphates by passage through a Sephadex G-25 column (1.5 x 40) equilibrated with the above buffer. Carbamylated and dimethyladipimidate modified hemoglobin were prepared as described by Williams, et al. (7) and Lockhart and Smith (8), respectively.

Inositol hexaphosphate (IHP) was dissolved in the buffer used for the spectral studies of the hemoglobin sample and adjusted to the appropriate pH by addition of concentrated HCl. The titrations of aquomet HbA and HbF were performed by addition of μl quantities of IHP to 1.0 ml of the hemoglobin sample in a 1 mm CD cell. The sample was allowed to equilibrate for 15 minutes after each addition and the peak height at 286 nm recorded over a period of 20 minutes. The average value of the peak height for each addition was then used to calculate $\Delta\varepsilon_{286}$. The CD spectra were recorded on a Jasco J 40-C recording spectropolarimeter as previously described (9). All CD studies were performed at room temperature with molar ellipticities reported in terms of tetramers.

Results

Figure 1 shows a comparison of the uv CD spectra of oxy and deoxy HbA and HbF. The most notable difference is the negative ellipticity seen for both oxy and deoxy HbF in the 280-290 nm region whereas only deoxy HbA is seen to possess negative ellipticity. Minor differences can also be readily seen in the number and positions of the uv CD bands of HbA and HbF. Oxy HbA is seen to possess two bands of weak positive ellipticity at 287 and 292 nm whereas oxy HbF possesses three bands of comparable negative ellipticity centered at 282, 285 and 290 nm. Deoxygenation of HbA was found to produce a single broad band of negative ellipticity centered at 286 nm whereas deoxygenation of HbF was found to produce two bands of negative ellipticity centered at 286 and 282 nm.



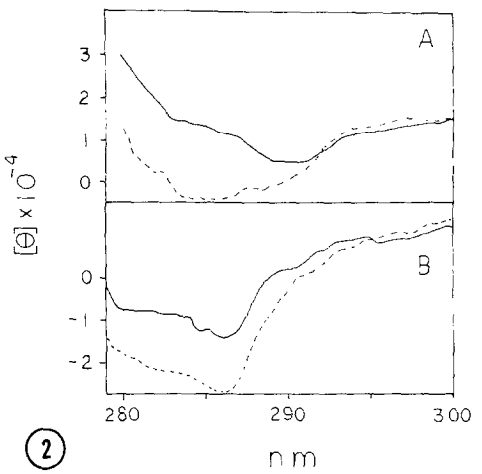


Figure 1 Circular dichroism spectra of oxy and deoxy HbA and HbF in 50 mm bis tris of pH 6.5. Hemoglobin concentration 60 x 10^{-6} M tetramer. A. HbA (——) oxy, (---) deoxy. B. HbF (——) oxy, (---) deoxy. Ellipticities are reported in terms of tetramers.

Figure 2 Circular dichroism spectra of stripped quomet HbA and HbF in the presence and absence of IHP buffered in 50 mm bis tris of pH 6.5. Hemoglobin concentration 60 x 10^{-6} M tetramer. A. HbA, (——) stripped, (---) stripped plus four moles IHP per mole hemoglobin tetramer. B. HbF (——) stripped, (----) stripped plus four moles IHP per mole hemoglobin tetramer. Ellipticities are reported in terms of tetramers.

Figure 2 shows a comparison of stripped aquomethemoglobins A and F in the presence and absence of IHP. Distinctive differences are also seen in the comparison of uv CD spectra of the adult and fetal hemoglobins. HbF even when stripped of organic phosphates was again found to possess three fairly distinct bands in its uv CD spectrum; a band of slight positive ellipticity can be seen at 290 nm (identical in position to that of normal hemoglobin) and two bands of distinctly negative ellipticity centered at 286 and ∿ 284 nm. This is in contrast to the positive ellipticity seen for HbA with two weak peaks centered at 286 and 292 nm. As expected the addition of saturating concentrations of IHP to either aquomethemoglobin A or F was found to produce changes in the ellipticity of the uv CD. In both

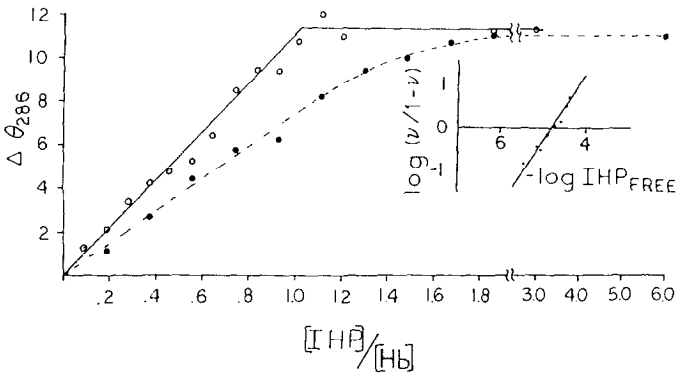


Figure 3 Change in ellipticity as a function of hemoglobin·IHP interaction. Hemoglobin concentrations 60 x 10^{-6} M tetramer buffered in 50 mM bis tris 0.1 M NaCl of pH 6.5. HbA (open circles),HbF (closed circles). Insert: Determination of the K_D for HbF·IHP complex (see text for details).

instances the major ellipticity change appeared in the 285 and 280 nm region with only a slight difference in the ellipticity of the 290 nm region. In particular, aquomet HbA changed from a slightly positive to a slightly negative ellipticity whereas aquomet HbF became more negative in ellipticity.

Figure 3 shows the results of the uv CD titration of stripped aquomet hemoglobins A and F as a function of IHP. The titration of aquomet HbA clearly shows that one mole of IHP was bound per tetramer of hemoglobin. A similar titration study with aquomet HbF shows a weakened interaction of IHP with HbF. The data were found to be non-stoichiometric with the direct determination of the binding constant of IHP to aquomet HbF obtained from Hill plots. Assuming a stoichiometry of one mole of IHP bound per mole of hemoglobin tetramer, a dissociation constant (K_D) of 16 x 10^{-6} M was obtained. The titration data also showed that the change in ellipticity upon binding saturating amounts of IHP was identical for both stripped aquomet hemoglobins A and F even though HbA goes from a positive to a slightly negative ellipticity and HbF is initially negative in ellipticity and proceeds to a more negative value.

In order to characterize the negative ellipticity seen in the uv CD region of HbF, chemical modifications using dimethyladipimidate (DMA) and

cyanate were performed. Modification with DMA significantly altered the uv CD spectrum of the aquomet HbF derivative but did not produce a spectrum similar to either native or DMA-modified met HbA. Although the modification of HbF with DMA decreased the negative ellipticity of HbF, the most prominent result was the lack of IHP effect on DMA-modified aquomet HbF. Carbamylation was found to be without effect on the uv CD spectrum of stripped aquomet HbF, but was found to inhibit the effect of IHP on the uv CD spectrum.

Discussion

In their circular dichroism studies Perutz and coworkers (10) implied that a change in the uv CD ellipticity of aquomet Hb from slightly positive to slightly negative upon addition of IHP could be ascribed to the R→T conformational change. Therefore, Wind et al. (4) interpreted the negative ellipticity of aquomet HbF at low pH as indicative of HbF more readily adopting the T conformation. This interpretation must be re-examined in light of the present data.

A comparison of the oxy, deoxy and aquomet uv CD spectra of hemoglobins A and F reveals that hemoglobin F possesses an inherently different uv CD spectrum. Aquomet HbF was found to exhibit ellipticity minima at 290, 286 and 284 nm. A close examination of the CD results reveals the spectra are similar except that the ellipticity is essentially shifted to negative values at 286 and 284 nm under all circumstances. This negative ellipticity is not the same as ascribed by Perutz and coworkers to be indicative of the R⇄T conformational change (9), but rather should be ascribed to a different origin.

The uv CD titration data of aquomet HbA clearly shows a one to one stoichiometry for the binding of IHP to hemoglobin. The titration data for aquomet HbF revealed a weakened interaction of IHP for HbF with a K_D value of 16 μM, identical to that obtained by Perutz and coworkers who monitored changes in the porphyrin environment by using visible absorption

spectroscopy (10). Our uv CD titration results coupled with the visible absorption titration data of Perutz and coworkers (10) for the first time clearly shows a parallel change in the porphyrin (visible) and amino acids (uv CD) residues and offers proof that uv CD spectroscopy is useful in monitoring the protein portion of the R⇄T conformational change. Of particular importance to this study was the finding that although stripped aquomet HbF possessed a negative ellipticity in the uv CD region, the magnitude of change in ellipticity upon addition of saturating concentrations of IHP was identical to that seen for aquomet HbA. These findings strongly suggest that the negative ellipticity seen for stripped aquomet HbF is not due to an increased tendency of HbF to form the T conformation but rather due to the tryptophan for tyrosine amino acid substitution at position 130 of the non-α chain. Further support that the negative ellipticity seen in stripped aquomet HbF is not due to the increased tendency to form the T conformation comes from chemical modifications which are known to cause an increased oxygen affinity and an altered uv CD spectrum upon addition of IHP (8). Under conditions of cyanate or dimethyladipimidate modification the negative ellipticity of aquomet HbF was still found to be present in the uv CD although the IHP effect was inhibited. This is in marked contrast to aquomet HbA in which these modifications do not give rise to a negative ellipticity upon IHP addition.

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